

Patent Claims

1. A method for the investigation of cytosine methylations in DNA sequences is hereby characterized in that
 - a) the DNA to be investigated is hybridized to oligonucleotides of a defined methylation status.
 - b) the hybrids are reacted with at least one hemi-methylation-sensitive restriction enzyme,
 - c) a detection is made of whether a restriction has occurred,
 - d) the methylation state of the investigated DNA is concluded.
2. The method according to claim 1, further characterized in that the oligonucleotides are bound to a solid phase.
3. The method according to at least one of the preceding claims, further characterized in that the oligonucleotides bear at least one detectable label.
4. The method according to at least one of the preceding claims, further characterized in that the oligonucleotides are labeled with a dye and a quencher, which are separated by a restriction.
5. The method according to at least one of the preceding claims, further characterized in that both methylated as well as unmethylated oligonucleotides of the same sequence are used simultaneously.
6. The method according to at least one of the preceding claims, further characterized in that the methylated and unmethylated oligonucleotides bear different labels.
7. The method according to at least one of the preceding claims, further characterized in

that several oligonucleotides of different sequence are used.

8. The method according to at least one of the preceding claims, further characterized in that the oligonucleotides are immobilized to a sensitive surface, whose physical or chemical properties are modified by a restriction in a way that can be measured.

9. The method according to claim 8, further characterized in that the modifiable properties include conductivity, characteristic frequency or surface tension.

10. The method according to at least one of claims 8 to 9, further characterized in that the surface involves a piezoelectric crystal.

11. The method according to at least one of the preceding claims, further characterized in that a restriction enzyme is used which cleaves unmethylated and hemi-methylated DNA preferably as opposed to homo-methylated DNA.

12. The method according to at least one of the preceding claims, further characterized in that the enzyme is taken from the following group: Acsil; Adel; Ascl; HinPI; Clal; Ecil; HinP1I; Hpy99I; Nrul; RsrlI; Sall.

13. The method according to at least one of claims 1 to 11, further characterized in that a restriction enzyme is used which cleaves unmethylated DNA preferably as opposed to hemi-methylated and homo-methylated DNA.

14. The method according to at least one of the preceding claims, further characterized in that several different restriction enzymes are utilized simultaneously or sequentially.

15. Use of the method according to claims 1 to 14 for the diagnosis of cancer disorders or other diseases associated with a change in the cytosine methylation status, for predicting undesired drug effects and for distinguishing cell types or tissues or for investigating cell differentiation.

16. Use of hemi-methylation-sensitive restriction enzymes for methylation analysis,

particularly for the diagnosis of cancer disorders or other diseases associated with a change in the cytosine methylation status, for predicting undesired drug effects and for distinguishing cell types or tissues or for investigating cell differentiation.

17. Use according to claim 16, further characterized in that one of the following restriction enzymes is used: Acsil; Adel; Ascl; HinPI; Clal; Ecil; HinP1I; Hpy99I; Nrul; RsrlI; Sall.
18. A test strip, on which oligonucleotides with a different methylation status and/or a different sequence are immobilized, and which permits restriction and detection in one step.
19. A kit, comprised of immobilized oligonucleotides, at least one hemi-methylation-sensitive restriction enzyme and the necessary reaction buffers.